

Color of Light Reflected to Leaves Modifies Nutrient Content of Carrot Roots

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ABSTRACT

Improved yield and nutrient content of food crops are important to both growers and consumers. We hypothesized that color of light reflected from the soil surface to developing leaves of field grown plants could result in modified concentrations of nutrients in edible roots. Carrot (*Daucus carota* L.) was used as the test plant. The plants were grown in trickle-irrigated field plots that were covered with panels that reflected various combinations of far-red (FR), red (R), and blue light (BL) to the growing leaves. The highest FR to R photon ratio reflected to developing leaves resulted in greatest shoot weight and the lowest root-to-shoot weight ratio. However, an increased quantity of photosynthetic light resulted in greater total weight per plant. Roots from yellow- and white-covered plots had highest concentrations of β -carotene and ascorbic acid. Those from yellow- and black-covered plots had highest concentration of phenolics. In general, concentrations were higher in cortex than in xylem tissues. We conclude that color of light reflected from the soil surface to developing leaves can influence yield and chemical composition of edible roots. This discovery suggests that color of light reflected to growing shoots may also influence chemical composition of plant species used as phytonutraceuticals.

NATURAL SOILS AND PLANT RESIDUES are of many colors, and they can reflect a wide range of photosynthetic and morphogenic light to influence yield and quality of growing plants. Photosynthetically active light is a well-known component of the growth environment, contributing to more than 90% of the dry matter through the photosynthetic process. However, use of morphogenic light is an emerging strategy in field crop production (Kasperbauer, 1992). It utilizes a plant's natural growth regulating system to regulate allocation and use of photosynthate within the developing plant. Much of this approach is based on results of controlled environment studies in which specific wavebands (colors) of light are used to modify gene expression in a way that can influence concentrations of end products that contribute to quality. The most influential colors of morphogenic light appear to be far-red (FR), red (R), and blue (BL).

Controlled environment studies of the 1960s and 1970s documented phytochrome involvement (as demonstrated by R-FR photoreversible control) in regulation of chlorophyll and carotenoid concentrations, photosynthetic efficiency, sugar and organic acid concentrations, and photosynthate allocation among developing parts of a plant (i.e., leaves, stems, and roots) (Kasperbauer, 1971; Kasperbauer and Peaslee, 1973; Kasperbauer et al., 1970). Also in the late 1960s, it was hypothesized (and later proven) that FR reflected from leaves of growing plants could alter the FR/R photon

ratio enough to act through the natural phytochrome system to modify development of nearby plants (Ballaré et al., 1987, 1990; Kasperbauer, 1971, 1987; Kasperbauer et al., 1984). Shortly thereafter, it was discovered that the effective light signal could also be reflected to growing plants from different colors of dead plant residue, natural soil, and painted panels on the soil surface (Hunt et al., 1989; Kasperbauer, 1992; Kasperbauer and Hunt, 1987, 1992). Reflection of morphogenic light from the soil surface allowed plants growing in full sunlight to receive a preselected light signal that would regulate the relative size of shoots and roots (Kasperbauer, 1992). For example, a red plastic mulch was developed to reflect a FR/R photon ratio that was higher than the ratio in incoming sunlight at the same time and place (Kasperbauer, 1999). It favored above-ground growth, including fruit yield of high value crops such as tomato (*Lycopersicon esculentum* Mill.) (Kasperbauer and Hunt, 1998) and strawberry (*Fragaria* \times *ananassa* Duch) (Kasperbauer, 2000). In addition to the higher yield of these two crops, the color of light reflected to the fruit during development also influenced concentration of some flavor and nutrient components in the ripe fruit (Kasperbauer et al., 2001).

Other colors of soil covers were used to reflect FR/R ratios that were lower than the ratio in incoming sunlight, and they favored below-ground growth (Kasperbauer, 1992). In a previous study with turnip (*Brassica rapa* L.), which is used as both a leaf and a root crop, we found that color of light reflected from the soil surface to leaves of sun-grown plants resulted in modified concentrations of sugars and glucosinolates in the edible roots (Antonious et al., 1996). An increased amount of blue light reflected to the leaves increased the sharp flavor and the concentration of glucosinolates (Antonious et al., 1996). That discovery was important because glucosinolates have been shown to reduce the risk of certain cancers in humans (Wattenberg, 1993). Hence, it was also important to determine whether concentrations of other nutrients and/or health-related compounds in edible roots of food crops could be affected by color of light reflected from the soil surface to leaves of sun-grown plants.

The objectives of our present study were to determine whether color of light reflected to growing carrot leaves could affect (i) shoot and root size, and (ii) concentrations of β -carotene, ascorbic acid, and phenolics in edible roots.

MATERIALS AND METHODS

Plant Materials and Growth Conditions

Carrot (cv. Scarlet Nantes) plants were grown in trickle irrigated field plots of Norfolk loamy sand (Typic Kandidults)

Abbreviations: BL, blue light; FR, far-red light; PPF, photosynthetic photon flux; R, red light.

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at the Coastal Plains Soil, Water and Plant Research Center near Florence, SC. Different colored panels on the soil surface were used to determine whether FR and visible light reflected from the soil surface to leaves of the growing plants could alter concentrations of β -carotene, ascorbic acid and phenols in the edible roots. Since our objective was to answer these questions rather than to maximize yield per hectare, the plant spacing pattern was designed to allow reflection from panels on the soil surface to developing leaves of the individual plants.

Each year, 90-cm-wide by 15-cm-high raised beds were prepared at 1.8-m intervals. Trickle irrigation tubes (with water emitters at 30-cm intervals) were placed on top of the beds and covered with 1.5-m-wide black plastic mulch. There were three such plots each year. Each plot contained 6-m subplots with different colored surfaces to provide different combinations of reflected BL, R, and FR. The sequence of color was randomized in each plot. Exterior white, blue, green, and yellow enamels were painted onto the plastic surfaces to provide some of the colors. Some plots were left unpainted (black) and others were covered with a red plastic that was formulated to reflect a wavelength combination that favored shoot crops such as tomato [manufactured by Sonoco Products Co., Hartsville, SC, and marketed as Selective Reflective Mulch (SRM-Red) by Ken-Bar, Inc., Reading, MA]. The colors used in 1994 to study effects of reflected color on shoot and root size were white, green, blue, and red. The same four colors plus yellow and black were used during the other years, 1996 through 1998. Exterior enamels were used because they provided an economical and repeatable method to obtain the desired reflection spectra for small plot studies. However, reflection spectra had to be measured from each batch of paint because two batches of a given color may appear identical to humans but quite different to plants if the paints reflect different amounts of FR. Each color of paint used in our study reflected the same spectrum each of the years.

Each year, plants were started in 11-cm-deep pots on a greenhouse bench the first week of April. A month later, they were selected for uniformity and hand set through 7.5-cm holes that were cut 45 cm apart in the plastic along the ridge of the raised beds. In this system the holes allowed heat to escape from below the plastic, and there was a high probability that all of the developing leaves received light reflected from the mulch color over which they were grown. The fleshy roots developed in the soil below the holes and leaves extended outward over the mulch surfaces. Soil temperatures near the developing roots of plants grown with, for example, blue versus green soil covers differed less than 0.5°C. Those grown with white soil covers were 1 to 2°C cooler. The plants were harvested in early July of each of the four years. In 1994, fresh weights of leaves and fleshy roots were obtained from each plant. During the other three years, fleshy roots were separated from the leaves and transferred to Kentucky State University for chemical analyses. Cortex and xylem tissues of five roots per color per replicate plot were separated and the samples were analyzed for β -carotene, ascorbic acid, and phenol concentrations. The same procedure was followed each of the three years. Separating the roots into cortex and xylem allowed us to determine whether concentrations differed in these tissues. Our rationale was that concentration difference between root components might provide important background for future development of procedures to grow plants of other species for content of phytonutraceuticals.

Reflected Light

The quantity and spectral distribution of upwardly reflected light were measured 15 cm above the various colored surfaces

with a LI-COR LI-1800 spectroradiometer (LI-COR Inc., Lincoln, NE) equipped with a remote hemispherical light collector on a 1.5-m fiber optic probe. Measurements were made at 5-nm intervals between 400 and 800 nm. A reference spectrum was obtained by measuring incoming sunlight at the same wavelengths. Light measurements were taken on a cloudless day at solar noon ± 30 min. The reflected light values were then calculated as percentages of incoming sunlight at each measured wavelength. The FR/R ratios received by leaves over the various colors included both reflected and incoming R and FR, and are expressed relative to the FR/R ratios in incoming sunlight. The rationale for this approach was that field plants normally grow in sunlight and they might be able to sense and respond chemically as well as morphologically to deviations in the spectra of light received by the growing leaves. That is, to a light environment that differs in spectral distribution from incoming sunlight.

Chemical Analysis

β -Carotene

After separating cortical tissue from xylem, 0.5-kg samples of each kind of tissue were cut into small pieces and 30-g representative subsamples were blended in a household blender at high speed with 100 mL of acetone for 2 min in dim light. The homogenate was filtered with suction through a Buchner funnel containing Whatman filter paper No. 1 (Fisher Scientific Company, Pittsburgh, PA). The resulting thick paste was extracted twice with acetone until the extract was colorless. The filtrates were combined, transferred to a separatory funnel containing 50 mL of 4% aqueous NaCl and 100 mL of petroleum ether. The procedure of extraction was completed according to the methods of Sweeney and Marsh (1970) and Antonious (1982). Absorption of the petroleum ether layer was measured at 450 nm in dim light. A calibration curve was prepared for each group of samples using 99% pure β -carotene (Fisher Scientific, Pittsburgh, PA) in the range of 1 to 10 $\mu\text{g mL}^{-1}$.

Ascorbic Acid

Representative samples of carrot roots (50 g) were blended with 100 mL of 0.4% oxalic acid solution for ascorbic acid extraction (Antonious, 1982). The homogenate was filtered through a Buchner funnel containing Whatman filter paper No. 1. Ascorbic acid was determined by the 2,6-dichlorophenol indophenol method (AOAC, 1970). L-Ascorbic acid of 100% purity (Sigma Chemical Company, St. Louis, MO) was used to establish a calibration curve.

Phenolics

Representative samples of carrot roots (20 g) were blended with 150 mL of ethanol to extract phenols. Following filtration through Whatman No. 1 filter paper, an aliquot of 1 mL was used for phenol determination. Soluble phenolic constituents were determined by Folin-Ciocalteu colorimetric method (McGrath et al., 1982). A standard calibration curve was obtained with pure chlorogenic acid (Fisher Scientific Company, Pittsburgh, PA) in the range of 1 to 16 $\mu\text{g mL}^{-1}$.

Statistical Analyses

Data were analyzed by analysis of variance as outlined by the SAS Institute (SAS, 1999). Duncan's Least Significant Difference test was used to compare means (Snedecor and Cochran, 1967).

Table 1. Characteristics of upwardly reflected light above the different colored soil covers relative to those of incoming sunlight at the same time and place, and FR/R ratios 15 cm above the soil surface.

Characteristic†	Color on soil surface					
	Black	White	Yellow	Red	Blue	Green
	%					
PPF (400–700 nm)	<5	39	33	12	12	11
Blue (BL) (450 ± 10 nm)	<5	38	12	5	20	6
Red (R) (650 ± 10 nm)	<5	40	39	24	8	10
Far-Red (FR) (745 ± 10 nm)	<5	39	40	28	12	14
	ratio					
FR/R‡	1.000	0.993	1.007	1.033	1.037	1.036

† PPF, Photosynthetic Photon flux. Values for reflected light are means of at least three measurements taken about 15 cm above the respective color, and expressed as percentages of incoming sunlight at the same wavelengths. Measurements were made at solar noon ±0.5 h on a cloudless day in June, 1994, at Florence, SC.

‡ FR/R photon ratios refer to the amounts reflected plus incoming and they are expressed relative to the ratios in incoming sunlight at the same time.

RESULTS

Reflected Light

The photosynthetic photon flux (PPF), BL, R, and FR values in upwardly reflected light (relative to those in incoming sunlight) measured about 15 cm above the different colors of soil covers as described in Materials and Methods are summarized in Table 1. The PPF values in reflected light were higher over the white and yellow surfaces than over the other colors. Therefore, the reflected (plus incoming) PPF values indicate that leaves developing in sunlight over white and yellow surfaces received substantially more photosynthetic light. They also reflected FR/R ratios that were very similar to the FR/R ratio in incoming sunlight during root development. On the other hand, leaves developing over the green, blue, and red surfaces received less reflected photosynthetic light but higher FR/R ratios. When both reflected and incoming R and FR were considered, the ratios over red, green, and blue surfaces were all $3.5 \pm 0.2\%$ higher than the ratio in incoming sunlight (see Table 1). This experimental system allowed comparing effects of increased photosynthetic light (over white and yellow) with low BL and increased FR/R photon ratios (over green and red). Additionally, a major difference in reflection from the white and yellow surfaces was that white reflected more BL. The blue surface reflected more BL than did the red and green surfaces, and the red surface reflected more R and FR than did the blue and green surfaces. Plant responses to color of reflected light are expected to become increasingly important in development of production systems for speciality crops grown for yield and concentration of compounds, such as antioxidants, that contribute to human health.

Shoot and Root Size

The largest roots, smallest shoots, and greatest total weight developed on plants whose leaves grew over the white surfaces (Table 2). Whereas, the leaf weight per plant and the shoot to root weight ratio were greatest with red soil covers, which reflected less BL and a higher FR/R photon ratio than was reflected by the white surface (see Table 1). Thus, total weight per plant over white was consistent with the greater amount of photosynthetic light reflected to the leaves. The smaller shoot to root weight ratio over white was also consistent with

the lower FR/R photon ratio reflected from the white surface relative to those reflected from the red, green, or blue surfaces. Although accurate measurements of effects of morphogenic light (primarily the FR/R photon ratio) on shoot/root photosynthate allocation in field-grown crops have been limited, the responses to PPF and FR/R with carrots are consistent with responses of other plant species in controlled-environment experiments with potted plants from which all roots could be recovered (Kasperbauer, 1971; Kasperbauer et al., 1984).

Plants that grew in field plots with the blue soil covers produced the lowest total weight per plant. Also, in a flavor preference test of carrots by 10 randomly selected persons, roots from plants that grew with blue soil covers were reported to be least desirable and those that developed with red were most desirable. Since the green, blue, and red soil covers used in our study all reflected higher FR/R ratios (relative to the ratio in incoming sunlight) and the blue surface reflected more blue light, our observations suggest that FR, R, and BL reflected to developing leaves may affect not only allocation of growth among shoots and roots (i.e., size) but also concentrations of compounds that influence flavor and nutrient content of edible roots. Thus, we conducted a 3-yr study to determine whether concentrations of β -carotene, ascorbic acid, and phenolics in carrot roots could be affected by color of light reflected from the soil surface to the developing leaves.

Chemical Responses

β -Carotene

The β -carotene content of roots from carrot plants whose leaves developed over the various colors of soil covers are presented in Table 3. Roots of plants grown

Table 2. Average fresh weight of carrot shoots and roots grown in trickle irrigated field plots covered with different colored soil covers near Florence, SC, in 1994.

Plant part	Color on soil surface			
	White	Blue	Green	Red
Shoot (g/plant)	57.8b†	65.2ab	72.0ab	79.0a
Root (g/plant)	158.8a	92.4b	103.2b	105.9b
Shoot/Root (ratio)	0.364b	0.706a	0.698a	0.746a

† Within each line, values followed by the same letter do not differ significantly at $P = 0.05$.

Table 3. β -Carotene concentrations (mg per kg fresh wt.) in cortex and xylem tissues of roots from carrot plants grown in field plots with different colored soil covers.

Tissue	Color on soil surface					
	Black	White	Yellow	Red	Blue	Green
Cortex	21.9b†	29.5a	28.0a	20.3bc	16.9c	17.9bc
Xylem	10.4b	14.6a	12.5ab	10.1b	11.3b	10.0b
Sig. ($P = 0.05$)	*	*	*	*	NS	*

* Indicates difference in values from cortex and xylem tissue within a given color were significant at $P = 0.05$, and NS indicates difference was not significant at $P = 0.05$.

† Values are means for the three year study. Within each line, values followed by the same letter do not differ significantly at $P = 0.05$.

with white and yellow had significantly higher concentrations of β -carotene than did those grown with the other colors. Both the white and yellow surfaces reflected more photosynthetic light and a lower FR/R ratio than the blue, green, and red surfaces. Other spectral differences in reflection from white and yellow (see Table 1) apparently had less influence than PPF and FR/R ratio on accumulation of β -carotene in the carrot roots.

Cortical tissues contained significantly ($P = 0.05$) more β -carotene than did the xylem tissues for all colors except blue (see Table 3). The differences in β -carotene contents among carrots grown with the different colors of soil covers were more pronounced in the cortical than in the xylem tissues, as might be predicted because of the relatively higher content of this compound in cortical tissue. Differences in concentration of β -carotene in the inner vascular tissue of roots grown with the different colors, while less pronounced, were similar to the trends among the different colors for cortical tissues.

Ascorbic Acid

Similar to the trends noted for β -carotene, concentrations of ascorbic acid were numerically higher in cortex than in xylem tissue; and carrots grown with leaves extended over white and yellow soil covers contained numerically higher amounts of ascorbic acid in both cortex and xylem tissues than did those grown with the other colors (Table 4). Only in the case of white and yellow, however, were concentrations in xylem tissues signifi-

cantly ($P = 0.05$) higher than those in roots grown over green, black, red, or blue. Cortex tissue of carrots grown with black, however, contained significantly less ascorbic acid than did those grown with any other color. Since the most apparent differences between spectra reflected from the black versus white (see Table 1) was that white reflected greater percentages of all of the measured wavelengths, the data suggest major contribution of the higher PPF (reflected plus incoming) over white.

Soluble Phenolics

Concentration of soluble phenolics in carrots grown with the different colors of soil covers are presented in Table 5. Carrots grown with yellow contained significantly higher concentrations in both cortex and xylem tissues than those grown with any of the other colors except black. As with β -carotene, when averaged across all colors, concentrations of soluble phenolics were higher in cortex than in xylem tissue.

DISCUSSION

The discovery that color of light reflected from the soil surface to leaves of a food crop can influence concentrations of β -carotene, ascorbic acid, and soluble phenolics in roots is important because roots of many plants are important sources of nutrients and other compounds with antioxidant, vitamin, or provitamin activity. Previously, we examined the effect of reflection from colored soil covers to growing leaves on the accumula-

Table 4. Ascorbic acid concentrations (mg per kg fresh wt.) in cortex and xylem tissues of roots from carrot plants grown in field plots with different colored soil covers.

Tissue	Color on soil surface					
	Black	White	Yellow	Red	Blue	Green
Cortex	119.2b†	208.4a	188.4a	179.9a	184.8a	173.3a
Xylem	114.6b	176.3a	180.0a	113.9b	124.8b	132.0b
Sig. ($P = 0.05$)‡	NS	NS	NS	NS	NS	NS

† Values are means for the three-year study. Within each line, values followed by the same letter do not differ significantly at $P = 0.05$.

‡ NS indicates values from cortex and xylem tissue within a given color were not significantly different at $P = 0.05$.

Table 5. Soluble phenolic concentrations (mg per kg fresh wt.) in cortex and xylem tissues of roots from carrot plants grown in field plots with different colored soil covers.

Tissue	Color on soil surface					
	Black	White	Yellow	Red	Blue	Green
Cortex	153.0a†	81.3c	171.9a	121.9a	131.2b	124.4b
Xylem	118.0ab	81.2c	133.0a	76.3c	110.4b	79.8c
Sig. ($P = 0.05$)	*	*	*	*	NS	*

* Indicates values from cortex and xylem tissue within a given color were significant at $P = 0.05$, and NS indicates the difference was not significant at $P = 0.05$.

† Values are means for the three-year study. Within each line, values followed by the same letter do not differ significantly at $P = 0.05$.

tion of glucosinolates, or mustard oil glycosides, in turnip roots (Antonious et al., 1996). These compounds are highly characteristic of the Brassicaceae. However, their distribution is very limited in other Angiosperms.

In the present study, we determined that light reflected to leaves of growing plants could also affect nutrients that are more commonly distributed in plants. For example, the carotenoids are of ubiquitous occurrence in higher plants. These compounds, while more typical of aerial plant parts than of below ground storage organs, are found in high amounts in the roots of many root crops such as the sweet potato [*Ipomoea batatas* (L.) Lam.] in addition to carrots. β -carotene is the most important of the carotenoids with provitamin A activity. Similarly, ascorbic acid, or vitamin C, is a common essential vitamin found in many vegetables.

The phenolics are also of widespread occurrence in higher plants. Phenolics, while not commonly considered essential in the human diet, are known to provide a number of benefits to human health. Many have anti-ascorbic effects, probably by protecting ascorbic acid from oxidation. By virtue of their antioxidant activity, they may play a role in the protection of cardiovascular health and prevention of certain cancers. In addition, other phenolics are weak estrogen mimics and may help to reduce the incidence of some sex-linked cancers.

Several studies on phytochemicals and dietary components as blocking agents of chemical carcinogenesis provided evidence that plant polyphenols and certain vitamins act as antioxidants having inhibitory effects against cancer (Ames et al., 1993). For example, ascorbic acid (Block, 1992; Daviglus et al., 1996), β -carotene (Chew et al., 1999; Daviglus et al., 1996), and polyphenols (Baltes, 2000; Toursel, 1998; Vinson et al., 1998) have been reported to play a protective role against cancer in humans.

In summary, results from the present study indicate that color of light reflected from the soil surface to the growing leaves of carrot plants can act through the natural growth regulatory system within the plants to influence allocation of new growth among shoots and roots. Although elucidation of biochemical pathways influenced by the different colored surfaces was beyond the scope of our study, the results indicate that the quantity and color of light received by the leaves can also affect the root concentrations of compounds that are important to flavor, nutrition, and various aspects of human health. We suggest that the photobiological action of reflected light can become a useful component in field production systems to improve quality control of plant products grown as phytonutraceuticals. Further studies are in progress.

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